Biology and genetics of viruses, importance in medicine

Viruses

- **non-cellular organisms** (according to some theories, viruses are not considered to be real living organisms, because they lack the ability of autonomous reproduction)
- 1000x smaller than bacterial cells
- consists of one type of **nucleic acid** (either DNA or RNA) and a number of **different proteins** specific for particular type of virus
- cause a broad range of **diseases** including **malignant transformation** and cancerous growth
- in evolution, they are an important means of horizontal gene transfer, which increases genetic diversity
- due to frequent mutations, viruses change their antigen composition (influenza virus) or even species specificity - e.g. SIV (Simian Immunodeficiency Virus) has probably mutated to HIV (Human Immunodeficiency Virus)
- viruses are parasites fully dependent on host cells (they haven’t own protein synthesis and metabolic apparatus and thus replicate and reproduce only inside of the host cells)
- outside of the host cell, viruses exist in the form of independent viral particles (virions)

Structure of virion

- **different size and shape** (helical, icosahedral or more complex) of virions specific for particular viruses
- visible in electron microscope
- **capsid:** the protein coat of virion consisting of a number of identical subunits (capsomeres), which form the new capsid via the process of self-assembly; capsid surrounds and protects the nucleic acid (viral genome) inside; capsid proteins are coded by virus
- **envelope:** some viruses (enveloped viruses) have, except of capsid, an extra envelope on the surface with structure similar to the cell membrane (lipid and protein bilayer); viruses acquire the envelope during the release from the host cell (exocytosis; budding); except of lipoproteins coming from host cell, the envelope contains also specific viral proteins (antigens)

Reproduction of viruses

- **adsorption** (attachment): virus binds the specific receptors on the surface of host cell
- **penetration:** virions penetrate to the host cell via endocytosis or via fusion with plasma membrane in case of enveloped viruses
- **uncoating:** capsid is digested inside by the cell lysosomes; the viral nucleic acid and viral proteins are released into cytoplasm
- **replication** (multiplication) and **expression** of viral genome: expression of "early" viral genes; replication of viral genome; expression of "late" viral genes; synthesis of viral structural proteins
- **maturation:** the assembly of new virions from single components
- release of new infectious virions:
  1. **lysis of host cell:** the host cell is killed (its membrane bursts)
  2. **exocytosis** (budding): enveloped viruses (e.g. HIV) are released by budding without necessarily killing the host cell; during this process the virus acquires its envelope
  3. **latent infection:** the host cell survives; viral nucleic acid replicates inside of recipient cell free or inserted in genom, but the host cell is not damaged; only small amount of virions can be released; equilibrium between host and parasite; the disease is not manifested

Classification of viruses

- classification of viruses according to the type of host cell:
  - **animal viruses:** cause a wide range of diseases in humans and animals
  - **plant viruses:** affect plants
  - **bacteriophages:** parasites of bacterial cells
- further criteria of viral classification refer to their genomes:
  1. type of nucleic acid representing the viral genome:
     - **RNA viruses** (majority of viruses)
     - **DNA viruses**
  2. **structure of nucleic acid:** linear (e.g. adenoviruses) or circular (e.g. polyomaviruses); segmented (e.g. influenza) or nonsegmented
  3. number of nucleic acid strands: single stranded or double stranded
     - if single stranded nucleic acid represents the genome, the single strand is either **positive-sense** (+) or **negative-sense** (-):
     - single stranded DNA (ssDNA): e.g. parvoviruses
     - single stranded RNA (ssRNA) (+): e.g. poliovirus, retroviruses
     - single stranded RNA (ssRNA) (-): e.g. influenza, rabies, stomatitis, ebola
     - double stranded DNA (dsDNA): e.g. adenoviruses, herpesviruses

    - double stranded RNA (dsRNA): e.g. rotaviruses
4. strategy of genome expression:
    - ssDNA: the complementary (-) strand is synthesized, than the transcription into mRNA follows
    - ssRNA (+): the first step of viral reproduction is translation; this viral RNA is infectious in itself and is directly used as mRNA
    - ssRNA (-): the first step is synthesis of (+) strand, which can function as mRNA
    - dsDNA: the first step is transcription into mRNA
    - dsRNA: the first step is transcription into mRNA

Retroviruses

- the special group of viruses using reverse transcription within reproduction cycle
- although the retroviral genome is ssRNA (+), the first step of their reproduction is the reverse transcription of viral ssRNA into dsDNA (the reaction is catalysed by viral reverse transcriptase, which is brought in viral capsid)
- during the second step, viral dsDNA (the product of reverse transcription) is integrated by viral integrase into some of the chromosomes of infected cell; the template ssRNA is degraded by ribonuclease
- **retroviral genome:**
    - represented by two identical molecules of ssRNA (+) carrying three genes: gag (capsid proteins), pol (reverse transcriptase and integrase) and env (envelope proteins)
    - the part of the retroviral RNA is also the signal for packaging of the genome into capsid, binding site for specific host tRNA (functions as primer for reverse transcriptase) and long terminal repeats (LTR) situated at both ends of RNA molecule (LTRs allow the reverse transcription of new DNA strand and carry regulatory elements controlling the expression)
- **provirus:** viral dsDNA integrated in the genome of host cell
- provirus is transcribed by nuclear RNA polymerase II into new viral ssRNA (+) functioning as both viral mRNA and genom for new viral particles budding out from the host cell (host cell usually survives); new viral particles ensure the horizontal transfer of viral genome
- endogenous provirus: retrovirus, which has integrated into chromosomes of germ line cells, its genome is passed on to a following generations as a part of host cell genome (vertical transfer)
- **reverse transcription** lacks the usual control by proofreading, retroviruses thus mutate very often and become quickly resistant to antiviral pharmaceuticals (the development of effective vaccines and inhibitors is therefore impeded)
- Retroviruses and malignant transformation:
    1. **acute transforming retroviruses:** induce a rapid tumor growth since they carry viral oncogenes (v-onc) directly in their genome (viral oncogene has usually its origin in cellular protooncogene which has become the part of viral genome and has thus lost its natural regulation); an example is RSV (Rous Sarcoma Virus)
        - **non-acute transforming retroviruses:** induce a slow tumor growth, since it doesn’t carry any viral oncogenes; the genome of such a virus is inserted into the host genome near some cellular protooncogene, whose expression increases significantly as it has got under the control of viral promoter or/and enhancer (the protooncogene becomes oncogene); since the insertion is a random process, this malignant transformation isn’t as fast as in case of acute retroviruses
- **examples of retroviruses:** HIV, HTLV (Human T-cell Leukemia Virus)

Bacteriophages

• bacteriophage is a virus that infects bacterial cells • specific structure: capsid, sheath and fimbriae, which allow the attachment of virus on the surface of bacterial cell (via specific bacterial receptors) • specific mechanism of penetration into the host cell: in case of bacteriophages, only the nucleic acid is transported (injected) into the host cell (thorough the hollow sheath), the rest of the virus remains outside • two alternative mechanisms of reproduction: - lytic cycle: "early" genes (regulatory proteins for replication and transcription of viral nucleic acid and for degradation of host DNA); "late" genes (structural proteins for capsid and sheath); host cell is killed, about 100-300 virions are released - lysogenic cycle: o after entering the host cell, the viral DNA is integrated into the bacterial chromosome at the place of att sequence (attachment), which is carried by both, viral and bacterial DNA o prophage: is the bacteriophage integrated in the bacterial chromosome; the latent form; bacterial cells carrying prophage within their genome (lysogenic bacteria) continue to live and reproduce normally, prophage is passed on the daughter cells for many generations as a part of bacterial chromosome o induction: in certain circumstances (UV radiation, chemicals), the prophage can release again and switch to the lytic cycle o temperate phages: bacteriophages able to reproduce via lysogenic cycle • the ability of bacteriophage to infect and lyse particular bacterial strain can be tested using agar plates: the lysed cells form brightened plaques, which are visible in the coherent layer of living bacteria

Application of viruses in science and medicine

- important in the field of life science; helped us to understand the basic mechanisms of molecular genetics (replication, transcription, RNA processing, translation, protein transport, immunology)
- in genetic engineering: viruses, including particularly bacteriophages (lambda, M13), used for the construction of cloning and expression vectors
- industrial production: expression of heterologous proteins by viruses is used for the production of various pharmaceutical proteins, vaccine antigens and antibodies
- gene therapy: still more experimental, not in routine practise yet
    - seems to be suitable especially for cancer and monogenic diseases

- gene therapy uses recombinant viruses, which have most of their viral genes replaced by the human gene of interest
- particularly retroviruses (able to integrate the gene of interest to the genom of affected cells) and adenoviruses are used for the construction of such recombinant viruses (vectors):
  - recombinant viruses carrying the functional copy of gene, which is damaged in the patient’s genom (experiments with the therapy of cystic fibrosis)
  - recombinant viruses carrying the gene which stimulates immunity or allows marking of affected (cancer) cells to be damaged by components of immune system
  - recombinant viruses that have been reprogrammed to kill cancer cells (oncolytic viruses)